Enviromental,Virulence,Antimicrobial and Molecular Characterization Studies *on Infective Bacterial Plaque Biofilm*

A Synopsis

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INTRODUCTION

Microbial biofilm are complex communities of bacteria and are common in the human body and in the environment. In recent years, dental plaque has been identified as a biofilm. The biofilm undergoes maturation, and the resulting pathogenic bacteria complex can lead to dental caries, gingivitis, and periodonitis.In addition, dental biofilm, especially sub gingival plaque in patients with periodonitis,has been associated with various systemic disease and disorders, including cardiovascular disease, diabetes mellitus ,respiratory disease, and adverse pregnancy outcomes (Joann, 2007).

Our mouth consisting of microbial biofilm are complex communities of bacteria. It has been estimated that there are over more than 600 different species prevalent with distinct subsets predominating at different habitats (Dewhrist *et al*.,2010). *Streptococcus mutans* species of oral *streptococci* live in the oral cavity. Each species has developed specific specialized properties for colonizing different oral sites and constantly changing conditions to fight competing bacteria and to withstand external challenges. Imbalances in the microbial biota can initiate oral disease. Under special condition, commensal *streptococci* can switch to opportunistic pathogens, initiating disease and damaging the host. Oral *streptococci* has both harmless and harmful bacteria.*"Mutans streptococci* "Are the most important bacteria associated with tooth decay. *Streptococcusmutans*,the microbial species most strongly associated with carious lesions, is naturally present in the human oral micro biota (Baron,1996).

Dental caries is a transmissible infectious disease in which *streptococcus mutans* plays the major role. As in many infectious diseases, colonization by pathogens is required before the disease can occur. *streptococcus mutans* are generally considered to be the principal etiological agent of dental caries (Russell,2008).There is a range of virulence factors important for the establishment of *Streptococcusmutans* in the complex microbial community of dental biofilm. Studies of the virulence factors of *Streptococcusmutans* and their correlation with species biodiversity are fundamental to understanding the role played by colonization by different genotypes in the same individual, and the expression of characteristics that may or may not influence their virulence capacity and survival ability under different environmental conditions. Studies using

phenotyping and/or genotyping methods strongly suggest that the mother is the major primary source of infection for children who carry *S.mutans* and/or *S.sobrinus* strains (2-10) and the saliva is the principal vehicle by which transfer of *Streptococcusmutans* may occur (Napimogo *et al*.,2005). However, detection of genotypes that are not found in children's mothers or other family members indicates that *S.mutans* and /or *S.sobrinus* may also be acquired from other sources. Furthermore, variability in transmission can be associated with children's individual susceptibilities, including the period defined as the window of infectivity, which was reported to occur earlier in Brazilian children, the number of erupted teeth, the emergence of molars; the presence of enamel hyperplasia sucrose consumption, the action of nonspecific factors of the salivary and mucosal immune systems; and immunological conditions in children (Klein *et al*., 2004).

The ability of bacteria to survive and persist in a given environment will depend, in part, on their inherent genetic plasticity, which determines their ability to respond to fluctuating local environmental condition or stresses. The micro biota resident in the oral biofilm is subjected to many variable environmental stresses, including the availability or lack of nutrients, acidic ph., and exposure to organic acids. The proportions of *Streptococcus mutans* and *lactobacilli* were elevated in the biofilm of caries –active subjects, while *Actinomyces naeslundii* isolates formed a significantly greater proportion of the microbiota in samples from caries –free subjects. These observations support the assertion that the biofilm samples from the two subject populations were exposed to different environments and, consequently, to different stresses (Paddock *et al*., 2015).In oral streptococci , acid tolerance correlates well with the pH optimum of the FATPase enzyme (Sturr and Marquis,1992).

In a recent study of young adults, Found a maximum of seven genotypes in subjects who had previous caries experience,(Redmo-Emanuelsson *et al*.,2003). The pathogenicity of the dental plaque biofilm is enhanced by the fact that in biofilm form,the component bacteria have increased resistence to antibiotics and other chemotherapeutic agents and are less able to be phagocytized by host inflammatory cells. Therefore,control of the dental plaque biofilm is a major objective of dental professionals and critical to the maintenance of optimal oral health (JoAnn,2007).Molecular characterization of strains from plaque isolates is senitive method for the detection of particular strain ,and may also be suitable for carrying out large scale studies on

the cariogenicity of isolated strains.Defects in stress-responsive genes have been also associated with biofilm impaired phenotypes (Baev *et al*., 1999; Lemos and Burne, 2002; Lemos *et al., 2001;Lis and Kuramitus,2*003).The search for novel antimicrobial agent is one of the current major concerns in medical research due to increasing cases of antibiotics resistence (Chitnis *et al*., 2000).

Although the basic principle of immune protection from dental caries caused by *Streptococcus mutans* has been established in pre-clinical studies, the effective application of this approach to human needs further refinements.

Objectives

1) To study collection of samples from dental plaque patients.
2) To study the isolate of bacterial flora from dental plaque patients.
3) Maintenance and pure culture of isolated bacterial flora causing dental plaque.
4) Comparative studies of plaque from caries active and free mouth patients.
5) To perform the pathogenicity test for virulence and non-virulence strains.
6) To study the effect of different environmental conditions on the growth of virulence strain of dental plaque.
7) To study Antibacterial studies of cow dung extract against virulence strains and comparision with standard antibiotics.
8) To study molecular characterization of virulence strains.

Review of Literature

Dental plaque is an example of a biofilm; it occurs natural and it supports the host in its defense against invading microbes. In health, the microbial constituent of dental plaque is diverse and remains relatively stable over time (microbial equilibrium). The predominant microorganisms choose host molecules (eg., salivary mucins and a neutral pH for growth. Under certain event, this microbial equilibrium can break down and disease such as caries can occur (PD,2009).The nonspecific oral dental plaque hypothesis proposed that the entire microbial community of plaque that accumulated on tooth surface and in the gingival crevice contributed to the growth of periodontal disease. Plaque bacteria produced virulence factors and noxious product that initiated inflammation challenged the host defense system, and resulted in the destruction of periodontal tissue. Under this hypothesis, the quantity of plaque was considered to be the critical factor in the development of periodontal disease. Thus, increase in the amount of plaque, as opposed to specific pathogenic microorganisms found in the plaque, were viewed as being first responsible for inducing disease and disease progression.(Theilade,1986).

Studies on the microbial etiology of various forms of periodontal support the specific plaque hypothesis, which proposes that only some microorganisms within the plaque complex are pathogenic. Despite the presence of hundreds of species of microorganisms in periodontal pockets, fewer than 20 are daily found in increased proportions at periodontal diseased sites. These specific virulent bacterial species activates the host's immune and inflammation responses that then causes bone and soft tissue destruction (Thom's*et al*.,2006).

Streptococcus mutant is a gram-positive organism that is the primary causative agent in the formation of dental cavities in human. Gram-positive bacteria are those that are marked dark-blue or violet by gram staining. This is kept on the physical properties of their cell walls, as opposed to gram negative bacteria, which cannot keep back the crystal violet stain. *Streptococcus mutans* is a facultative anaerobic, Gram positive *coccus* -according to scientific classification (Ryan.,2004).

The microbe was first described by J Kilian Clarke in 1924. In areas associated to oral health care, bacterial biofilms are recovered in dental unit water lines, on tooth surfaces and oral dental prosthetic appliances or materials, and on oral mucous membranes.Plaque of supragingival and

subgingival are the biofilm which is the etiologic agent in dental caries and periodontal diseases. The pathogenicity of the *Streptococci* group bacteria is hugely critical to understand in oral biofilm.

The pathogenicity of plaque and *Streptococcus mutans* belonging to lactic acid group bacteria, a member of human oral flora, is generally recognized as the vital etiological agent of dental cavities, *Streptococcus mutans* tolerate rapidly harsh environment fluctuation and exposure to various anti-microbial agents in order to live. However, the biological process under which this cariogenic pathogen can live and proliferate in such diverse environmental conditions are largely unknown, as little research has been done on this matter (Baron, 1996).

Our mouth consisting of microbial biofilm are complex communities of bacteria. It has been estimated that there are of saliva from more than 600 different species. *Streptococcus mutans* species of oral *streptococci s*urvive in the oral cavity. Each species has developed peculiar specialized properties for colonizing different oral sites and repeatedly changing conditions to grapple competing bacteria and to withstand external challenges. Imbalances in the microbial biota can initiate oral disease. Under special condition, commensal *streptococci* can switch to opportunistic oral dental pathogens, cause disease and distruct the host .Oral *streptococci* has both harmless and harmful bacteria. *"Mutans streptococci* "Are the most principal bacteria associated with tooth decay. *Streptococcus mutans,*the microbial species most strongly associated descibed with carious lesions, is normaly present in the human oral micro biota (Cornejo *et al*.,2012).

A bacteria biofilm is often the cause of dental caries, persistent infection and has been associated with osteomyelitis, pneumonia in patient with cystic fibrosis and prostatitis. Talking about dental plague is typically the precursor to tooth decay and contain more than 600 different microorganisms contributing to the oral cavities over all changing environment that frequently undergoes rapid change in pH., nutrient availability and oxygen tension. Dental plague adheres to the teeth and consists of bacterial cells while plague is the biofilm on the surface of teeth (Baron,1996).

Dental caries is a transmissible and extremly infectious disease in which *Streptococcus mutans*. Plays the major role. As in many infectious and transmissible diseases, colonization by pathogens is required before the disease can occur. *Streptococcus mutans* are mainly considered

to be the principal etiological agent of dental caries.There is a range of virulence factors important for the establishment of *Streptococcus mutans* in the complex microbial community of dental biofilm. Studies of the virulence factors of *Streptococcus mutans* and their correlation with species biodiversity are important fundamental to understanding the role played by colonization by distinct genotypes in the same individual, and the expression of characteristics that may or may not influence their pathogenic or virulence capacity and survival ability under different environmental conditions. Studies using phenotyping and/or genotyping methods stronglysuggest that the mother is the major primary source of infection for children who carry *Streptococcus mutans* and/or *Streptococcus sobrinus* strains (2-10) and the saliva is the main vehicle by which transfer of may occur (Jeffrey, 2004) 50 years, the understanding and characterization of dental plaque have undergone significant changes (Thoms, 2006).

Structures of isolates and gross DNA composition confirmed the serological findings that there was prominent variation amongst the huge number of isolates identified as *Streptococcusmutans*. Based on a whole analatical raft of studies, the *Streptococcus mutans* isolates were sub-divided into a number of different species some of which were of animal and some from human sources. Thus the*"mutans streptococci*" were born and the name *Streptococcus mutans* was keep back to describe the more common of the two main human strains, the other being called *Streptococcussobrinus.* The retention of the name *Streptococcus mutans* has led to some confusion but was necessary to comply with the rules that governing scientific nomenclature (Nicolas*et al.*, 2011*).*

Dental caries is a infective biofilm plaque-related oral disease associated with increased consumption of dietary sugar and fermentable carbohydrates. When dental biofilms remain on tooth surfaces, along with constant exposure to sugars, acidogenic bacteria (members of dental biofilms) will metabolize the carbohydrates to organic acids. Persistence of this acidic condition encourages the formation of acidogenic and aciduric oral dental bacteria as a result of their ability to survive at a low-pH environment. The low-pH environment in the dental biofilm matrix destroys the surface of the teeth and begins the "initiation" of the dental caries (Hajishengallis and Russell 2008). If the adherence of *Streptococcus mutans* to the surface of oral teeth or the physiological potential of (acidogenity and aciduricity) of *Streptococcus mutans* bacteria in oral dental biofilms plaque can be reduced or eliminated, the acidification

conceivablity of dental biofilms plaque and later cavity formations can be decreased (Hajishengallis and Russell, 2008). It is widely accepted that the advent of agriculture in early human populations provided the conditions *Streptococcus mutans* needed to evolve into the virulent bacteria it is today. Agriculture introduced fermented foods, as well as more carbohydrate rich foods, into the diets of historic human populations. These new foods introduced new bacteria to the oral cavity and created new environmental conditions. For example, *lactobacillus* or *leuconostoc* are typically found in foods such as yogurt and wine. Also, consuming more carbohydrates increased the amount of sugars available to *S.mutans* for metabolism and lowered the pH of the oral cavity. This new acidic habitat would select for those bacteria that could survive and reproduce at a lower pH (Cornejo *et al.*,2012).

Another significant change to the oral environment occurred during the industrial revolution. More efficient refinement and manufacturing of foodstuffs increased the availability and amount of sucrose consumed by humans. This provided Streptococcus mutans with more energy resources, and thus exacerbated an already rising rate of dental caries (Hoshino *etal.*,2012) Refined sugar is pure sucrose, the only sugar that can be converted to sticky glucans, allowing bacteria to form a thick, strongly adhering plaque (Darlington,1979).

Due to the role the *Sreptococcus mutans* plays in tooth decay, there have been many attempts to make a vaccine for the organism. So far, such vaccines have not been successful in humans. Recently, proteins involved in the colonization of teeth by *S.mutans* have been shown to produce antibodies that inhibit the cariogenic process. A molecule recently synthesized in yale university and university of Chile (Klein *et al*.,1998). Called Keep 32 is supposed to be able to kill *streptococcus mutans*. [Surviving in the oral cavity *S.mutans* is the primary causal agent and the pathogenic species responsible for dental caries specifically in the initiation and development stages (Biswas *et al*.,2011).

While *Streptococcus mutans* grows in the biofilm, cells maintain a balance of metabolism that involves production and detoxification. Biofilm is an aggregate of microorganisms in which cells adhere to each other or a surface. Bacteria in the biofilm community can actually generate various toxic compounds that interfere with the growth of other competing bacteria. However, there have been very few studies on how *S.mutans* can tolerate such exposure to various toxic

substance during its growth in the oral biofilm and is,thus,poorly understood.(Aspiras *et al*.,2004).

In areas related to oral health care, bacterial biofilms are found in dental unit water lines, on tooth surfaces and dental prosthetic appliances, and on oral mucous membranes. Biofilm in the form of supragingival and subgingival plaque is the etiologic agent in dental caries and periodontal diseases. The pathogenicity of the dental plaque biofilm is enhanced by the fact that in biofilm form, the component bacteria have increased resistance to antibiotics and other chemotherapeutic agents and are less able to be phagocytized by host inflammatory cells. Therefore, control of the dental plaque biofilm is a major objective of dental professionals and critical to the maintenance of optimal oral health. Over the past 50 years, the understanding and characterization of dental plaque have undergone significant evolution (Banas *et al*.,2007).

Lower part of gut of the cow contain various microorganism including *Lactobacillus plantarum, Lactobacillus casei, Lactobacillus acidophilus, B. subtilis, Enterococcus diacetylactis, Bifido bacterium* and *yeasts* (commonly *Saccharomyces cerevisiae*) having probiotic activity.Normally aged cow dung gets invaded with several soil contaminants such as *bacteria,fungi,Trichoderma* and *Actinomycetes.* There are several evidence to show that the fresh cow dung and cow urine are antifungal and antiseptic in nature (Ware *et al.*,1988).

Materials and methods

1. Collection of plaque samples from dental unit of government hospital Daman (U.T).A comprised study of 500 samples which includes 200 male,200 female, and 100 children ranging from 18 to 60 years and children from 5 to 16 years as per the WHO guideline.
2. The nature of the work followed in this study will be fully explained to all participants and the study will be conducted with written informed consent.
    - The subject will be screened using an exploratory survey and who volunteered in the study will interviewed using a questionnaire.
    - Qualified subjects should not have any chronic disease or had not received antibiotic therapy for at least 7 weeks.
    - The clinical examination will be conducted by trained dentist to assess intra-examiner reliability.
    - Dental plaque collection: Sterile Tongue Depressor will be used to avoid contamination from other mouth parts and to aid a better vision of carious lesions.
3. Plaque sampling sited varied depending on the condition of the oral cavity. The plaque samples will be collected from carious lesions sites with sterile disposable swab stick.
    - Aseptically transfer of plaque into 1 ml of sterile phosphate buffer saline and stored at 4°C
    - Bacterial isolation from dental plaque by using different conventional media i.e mitis-Salivarius agar (hi media) will modified to MSB agar (Gold *et al*.,)adding 20% sucrose (Hi media) and 0.2 units /ml bacitracin (Hi media),mitis salivarius kanamycin-bacitracin (MSKB), glucose-sucrose-tellurite-bacitracin(GSTB),trypticase soy-sucrosebacitracin(TYS2OB)andtryptone-yeast-cysteine-sucrose-bacitracin(TYCSB) agar can be used.
    - The sample will be vortexed and plated on MSB agar followed by anaerobic incubation for 37°C at 48 hrs.
4. Isolated strain will be identified on the basis of its colonial morphology and biochemical test.
5. Hydrolytic enzyme and Acidogenicity test for detecting pathogenic characteristics of isolated strains.

6. Oxidative, Acid, Osmotic stress test through disk diffusion assays for environmental factors on isolated strains.
7. Antibiotic Sensitivity studies (Bauer *et al*.,1966).The inoculum of the isolates will be prepared by streaking the organisms on nutrient agar plates to obtain discrete colonies. From the nutrient agar plate, bacterial colonies will be transferred into McCartney bottles containing sterile normal saline to obtain bacterial density by McFarland standard scale number. The culture will be streaked uniformly onto freshly prepared Muller Hinton agar plates.
8. Further molecular characterization of isolated strain by using PCR technique in laboratory comparing the conventional method with species-specific PCRfor isolated strain in plaque sample.
9. Data will be tabulated and analyzed statistically.

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great

FROM:SHAILJA S SINGH

PROJECT SYNOPSIS FOR P.hd IN MICROBIOLOGY

TITLE: BIOFILMS SRENTHENS THE S.MUTANS IN THEIR CHARACTERISTICS OF TRANSMISSION AND TO SURVIVE IN HARSH ENVIROMENT.

INTRODUCTION: Our mouth consisting of microbial biofilm are complex communities of bacteria.

It has been estimated that there are of saliva from more than 600 different species. A bacteria biofilm is often the cause of persistent infection and has been associated with osteomyelitis, pneumonia in patient with cystic fibrosis and prostatitis. Talking about dental plague is typically the precursor to tooth decay and contain more than 600 different microorganisms contributing to the oral cavity's over all dynamic enviroment that frequently undergoes rapid change in ph, nutrient avilability and oxygen tension . Dental plague adheres to the teeth and consists of bacterial cells while plague is the biofilm on the surface of teeth .

Dental plague and lactic acid bacteria group S.mutans, a member of human oral flora , is widely

Recognized as the main etiological agent of dental cavities, S . mutans tolerate rapidly harsh

Enviroment fluctuation and exposure to various anti-microbial agents in order to survive.

However ,the mechanisms under which this cariogenic pathogen can survive and proliferate

In such extreme enviromental conditions are largely unknown, as little research has been done on this matter.

LITERATURE REVIEW

Sreptococcus mutans is a gram-positive organism that is the primary causative agent inthe formation of dental cavities in human. Gram-positive bacteria are those that are stained dark-blue or violet by gram

Staining. This is based on the physical properties of their cell walls, as opposed to gram – negative bacteria,which cannot retain the crystal violet stain. Streptococcus mutans is a facultatively anaerobic,

The lactic acid bacteria group. S.mutans,a member of the human oral flora,is widely recognized as the main etiological agent of dental cavities.

Conditions in oral cavity are diverse and complex ,freguently changing from one extreme to another .thus,to survive in the oral cavity ,S.mutans must tolerate rapidly harsh enviroment fluctuations and exposure to various anti-microbial

Agents in order to survive . however,the mechanisms under which this cariogenic

Pathogen can survive and proliferate under such extreme enviromental conditions are largely unknow ,as little research has been done on this matter.

Twenty-five species of oral streptococci live in the oral cavity. Each species has developed specific specialized properties for colonozing different oral sites and constantly changing conditions to fight competing bacteria and to withstand external challenges. Imbalances in the microbial biota can initiate oral disease.under special condition ,commensal streptococci can switch to opportunistic pathogens, initiating disease and damaging the host .Oral streptococci has both harmless and harmfull bacteria.“Mutans streptococci “

Are the most important bacteria associated with tooth decay.S.mutans,the microbial species most strongly associated with carious lesions,is naturally present in the human oral microbiota .the taxonomy of these complex bacteria remains tentavie. A 1970's study found that S.mutans was more prevalent on the pits and fissures ,constituting 39% of the totalstreptococci in the oral cavity. FewerS.mutans were found on the buccal surface.(2-9%) early colonizers of the tooth surface are mainly Neisseria spp. And streptococci ,including S.mutans .

The growth and metabolism of these pioneer species changes local enviromental conditions(e.g.,Eh.Ph ,coaggregation, and substrate availability),thereb enabling more fastidious organisms to further colonize after them,forming dental plaque.

Along with S.sobrinus,S.mutans plays a major role in tooth decay,metabolizing sucrose to lactic acid using the enzyme Glucansucrase. The acidic enviroment created in the mouth by yhis process is what cause the highly mineralized tooth enamel to be vulnerable to decay.S.mutans is one of a few specialized organisms

Equipped with receptors that improve adhesion to the surface of teeth . Sucrose

Is used by S.mutans to produce a sticky, extracellular ,dextran based polysaccharide that allows themto cohere,forming plaque. S.mutans produce dextran via the enzyme dextransucrase (a hexosyltransferase) using sucrose as a substrate in the following reaction:

n sucrose – (glucose)n +n fructose

sucrose is the only sugar that S.mutans can use to form this sticky polysaccharide.

However,many other sugars –glucose,fructose ,lactose can be digested by S.mutans ,but they produce lactic acid as an end –product .it is the combination of plaque and acid that leads to dental cavities.dental caries is a dental biofilm related oral disease associated with increased consumption of dietary sugar. When dental biofilms remain on tooth surfaces, along with frequent consumption of sugar acidogenic bacteria (member of dental biofilms) will metabolize the sugar to organic acids. Persistence of this acidic condition encourages the proliferation

Of acidogenic and aciduric bacteria as a result of their ability to survive at a low

Ph enviroment . the low –P H enviroment in the biofilm matrix erodes the surface of the teeth and begins the "initiation" of the dental caries. If the adherence of S.mutans in dental biofilms

can be reduced or eliminated,the acidification potential of dental biofilms and later cavity formation can be decreased.

Susceptibility to disease varies between individual and immunological mechanisms have been proposed to confer protection or susceptibility to the disease. These mechanisms have yet to be fully elucidated but it seems that while antigen presenting cells are activated by S.mutans in vitro ,they fail to respond in vivo. Immunological tolerance to S.mutans at the mucosal surface may make individuals more prone to colonisation with S.mutans and therefore increase susceptibility to dental caries.

S.mutans is acquired in the oral cavity at the moment of tooth eruption. But -S.mutans has been detected in the oral cavity of predentate children. This suggest

That the eruption of teeth is not a necessary prerequisite . thus ,this species may not be confined to dental plaque. The adhesion,invasion,and persistence within the oral cells are cosidered the virulence mechanism of S.mutans to colonize and survive in the oral cavity in the absence of a tooth surface.

S.mutans is implicated in the pathogenesis of certain cardiovascular diseases. S.mutans is the most prevalent bacterial species detected in extirpated heart valve tissues as well as in atheromatous plaques, with an incidence of 68.6% and 74.1% respectively.

Due to the role the S.mutans plays in tooth decay ,there have been many attempts to make a vaccine for the organism. So far, such vaccines have not been successful in humans. Recently, proteins involved in the colonozation of teeth by S.mutans have been shown to produce antibodies that inhibit the cariogenic process. A molecule recently synthesized in yale university and university of Chile

Called Keep 32 is supposed to be able to kill streptococcus mutans.

Surviving in the oral cavity Smutans is the primary causal agent and the pathogenic species responsible for dental caries specifically in the initiation and development stages.

Dental plaque is typically the precursor to tooth decay and contains more than 600 different microorganisms, contributing to the oral cavity’s overall dynamic

Enviroment that frequently undergoes rapid changes in ph ,nutrients availability,and oxygen tension.dental plaque adheres to the teeth and consists of bacterial cells while plaque is the biofilm on the surfaces of the teeth. Dental plaque and S. Mutans is frequently exposed to "toxic compounds" from oral healthcare products,food additives,and tobacco. Degradation by-products of dental composites resins can be another source of toxic chemicals that can interfere with the bacterial growth of S.mutans.

While Smutans grows in the biofilm, cells maintain a balance of metabolism that involves production and detoxification .biofilm is an aggregate of microorganisms in which cells adhere to each other or a surface. Bacteria in the biofilm community can actually generate various toxic compounds that interfere with the growth of other competing bacteria. However ,there have been very few studies on how S.mutans can tolerate such exposure to various toxic sucstance during its growth in the oral biofilm and is,thus,poorly understood.

Smutans has over time developed strategies to successfully colonize and maintain a dominant presence in the oral cavity. The oral biofilm is continuously challenged by changes in the enviromental conditions. In response to such changes, the becterial community evolved with individual members and their specific functions to survive in the oral cavity .S.mutans has been able to evolve from nutrition-limiting conditions to protect itself in exterme conditions . Streptococci represents 20% of the oral bacteria and actually determines the development of the biofilms. Although S.mutans can be antagonized by pioneer

Colonizers,once they become dominant in oral biofilms,dental caries can develop and thrive.the etiological agent of dental caries is associated with its ability to metabolize various sugars,form a robust biofilm,produce an abundant amount

Of lactic acid,and thrive in the acid enviroment it generates.

Microbialbiofilms are complex communities of bacteria and are common in the human body and in the enviroment .in recent years,dental plaque has been identified as a biofilm ,and the structure ,microbiology,and pathophysiology of dental biofilms have been described.the nature of the biofilm enhance the component bacteria's resistance to both the host's defence system and antimicrobials.if not removed regularly,the biofilm undergoes maturation,and the resulting

pathogenic bacterial complex can lead to dental caries,gingivities and periodonities. In addition,dental biofilm,

Especially subgingival plaque in patients with periodontitis ,has been associated with various systemic diesease and disorders,including cardiovascular disease ,diabetes mellitus,repiratory disease and adverse pregnancy outcomes.